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Extraction and Modification of Sorghum Starch and its Characterization

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ABSTRACT

Sorghum is a widely cultivated cereal crop that has the potential to be an excellent source of starch. Starch extracted from sorghum has been gaining attention due to its unique properties and its potential applications in various industries, including food, paper, and textile. Modified starch has been found to improve the functional properties of starch, such as increased water holding capacity, better stability, and improved gelling properties. The hydrothermal modification (HMT) method has been reported to be an effective technique for modifying the properties of starch. In this study, we aimed to extract and modify sorghum starch using the HMT method and evaluate its properties, including moisture content, ash content, fat analysis, protein content, and water holding capacity. The findings of this study will contribute to a better understanding of the potential use of sorghum starch in various industries and highlight the potential of the HMT method as a tool for modifying starch properties.

***KEYWORDS**: modified starch; functional properties; millets; hydrothermal; starch properties*

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INTRODUCTION

Sorghum bicolor (L.) Moench is a cereal crop of Poaceae family. It is an important crop in many regions of the world, particularly in Africa and Asia, where it is used for food feed, fuel, and industrial applications. Sorghum is a highly adaptable crop that can grow in a wide range of environmental conditions, making it an attractive option for farmers in marginal areas. It is the fifth most important cereal crop in the world The grain of the sorghum plant is rich in carbohydrates, protein, fibre, and minerals, and can be milled to produce flour for various food applications. Sorghum has been studied as a source of starch, which has unique properties such as high viscosity and gelatinization temperature, making it attractive for various industrial applications, such as in food, paper, and textile industries [1]. Food packaging is contributing huge environmental load by means of carbon emission. To address these environmental concerns, there is a growing need for alternative packaging materials that are biodegradable, compostable, and sustainable. Researchers are exploring various options, including natural biopolymers, such as starch, cellulose, and protein-based materials, as well as synthetic biodegradable polymers, such as polylactic acid (PLA) and polyhydroxyalkanoates (PHA) [2]. Starch-based materials have been proposed as an alternative to conventional plastic packaging due to their renewability, biodegradability, and availability as a by-product of the food industry.

Cereal grains are the potent source of polysaccharide in the form of starch. It is composed of two types of molecules: amylose and amylopectin. Amylose is a linear molecule, while amylopectin is highly branched [3]. The starch was modified in current study and certain parameters were studied of the starch. The modified sorghum starch was analysed for various proximate parameters, including water holding capacity, moisture content, ash content, fat analysis, and protein content. The results showed that the modified sorghum starch had a higher water holding capacity than the unmodified starch which is an important parameter as water holding capacity

affects many functional properties of starch. Additionally, the modified starch had a lower ash content, which could be beneficial for certain applications and it also indicates that the starch sample is of high quality and extraction was carried out properly. the modified starch had a lower protein content and higher fat content than the unmodified starch. This result can be significant in various applications of sorghum starch, such as in the food industry, where modified starch is often used as a thickening agent. A higher fat content can improve the texture and mouth feel of the food product, while a lower protein content can reduce allergenicity and improve digestibility. modified starches with a higher fat content have better emulsifying properties, making them suitable for use in products such as salad dressings and sauces (21). Sorghum starch is often used in gluten-free bread as a substitute for wheat flour, and a lower protein content in the modified starch may result in a weaker gluten network, leading to inferior bread quality [4]. Overall, the analysis of fat and protein content provides important information about the properties of modified sorghum starch and its potential applications in various industries. The study demonstrates that the HMT method can be successfully used to extract and modify sorghum starch to improve its functional properties, which could have important implications for the food industry.

MATERIALS AND METHODS

The research was carried out in the Department of Food Technology, Parul Institute of Applied Sciences, Parul University, Vadodara. This section enlists the material used and elaborates on the methodologies used during the research.

Materials

Raw materials

This whole study was completely based only on the starch extracted from a single source i.e., Sorghum. The sorghum required for this study was bought from the local wholesale market of Vadodara. The market is locally known as the "Khanderao market". The reason for buying raw material from the market for study instead of buying authenticate Sorghum was its intended future use.

Chemicals

Folin reagent, standard BSA solution, petroleum ether, 2.5N NaOH solution and distilled water.

Equipment and glassware

Equipment's and glassware's required for this study were muffle furnace, Soxhlet apparatus, test tubes, colorimeter, centrifuge machine, beakers, hot air oven and mixer.

Extraction of starch

NaOH extraction method is a widely used method for starch extraction. This method involves the use of sodium hydroxide (NaOH) solution to break down the protein and lipid complexes in the grain, which allows for the isolation of starch. The NaOH method is commonly used because it is simple, cost-effective, and results in a high yield of starch [6]. The Sorghum grains were dipped overnight in 2.5N NaOH solution. The grains were then washed with distilled water and were grinded using a mixture. The paste so formed was passed through a sieve and the extract was collected. The collected extract was then centrifuged at 4000 rpm for 15 minutes. The supernatant was discarded and the upper brown layer of aliquot was scrapped off. 10 ml distilled water was added to the tube and was mixed thoroughly by vigorous shaking. This procedure was repeated for three times and then the final aliquot was taken in the petri dish and was kept for drying in hot air oven at 50ºC.

Proximate analysis

Protein content

One of the widely used methods to determine the protein content of sorghum starch is the Lowry method. This method is based on the reaction between protein and cupric ions under alkaline conditions, which results in the formation of a blue-violet complex that absorbs light at 750 nm. Preparation of a protein standard curve using bovine serum albumin (BSA), preparation of sample solutions by dissolving the sorghum starch in distilled water and diluting the solution to a known concentration, addition of a reagent mixture containing copper sulfate, sodium carbonate, and sodium potassium tartrate to the sample solution, and incubation of the mixture at room temperature for 10-30 minutes, followed by addition of the Folin-Ciocalteu reagent and incubation for another 30 minutes. The absorbance of the resulting solution is then measured at 750 nm using a spectrophotometer, and the protein content of the sample is determined by comparing the absorbance with the standard curve. Several studies have used the Lowry method to determine the protein content of sorghum starch [7,8].

Fat analysis

The Soxhlet extraction method is commonly used to determine the fat content in various food products, including starch. The method involves extracting the fat from a sample using a solvent and then determining the amount of fat in the extract using soxhlet method. Weigh about 2-3 grams of sample using an analytical balance and record the weight. Place the sample into a thimble. Add 20-25 mL of petroleum ether to the Soxhlet extractor and heat the

flask using a heating mantle. Place the thimble into the Soxhlet extractor and set up the Apparatus. Allow the extraction to proceed for 6-8 hours or until the solvent in the flask becomes clear. Remove the thimble from the Soxhlet extractor and dry it in an oven at 100-105°C for 30 minutes. Weigh the dried thimble and record the weight. Calculate the fat content of the sample using the following formula:

$$\text{Fat content (\%)} = [(W_2 - W_1)/W_3] \times 100$$

Where, W1 = weight of empty thimble; W2 = weight of thimble with extracted fat; W3 = weight of sample taken

Ash content

The ash content of starch can be determined using a muffle furnace. The muffle furnace is a device that heats a sample to a high temperature in an oxygen-free environment, causing it to combust and leaving behind the inorganic residue. The ash content of the sample is then calculated by weighing the residue and comparing it to the initial weight of the sample. Weigh a clean and dry crucible and record its weight as W1. Add a known weight of the sorghum starch sample to the crucible and record the total weight as W2. Place the crucible in a muffle furnace and heat it at a temperature of 550-600°C for 3-4 hours until all the organic matter is burnt off and a white ash remains. Allow the crucible to cool in a desiccator for 30 minutes. Weigh the crucible with the ash residue and record the weight as W3. The ash content of the sorghum starch sample can be calculated using the following formula;

$$\text{Ash content (\%)} = (W_3 - W_1) / (W_2 - W_1) \text{ x } 100$$

where: W1 = weight of empty crucible; W2 = weight of crucible with sample; W3 = weight of crucible with ash residue.

Moisture content

Moisture content in starch is an important parameter to determine the quality of the starch product. The moisture content can be determined using hot air oven method. This method is based on the principle of drying the sample at a specific temperature for a specific time period to remove moisture from the sample. The weight loss of the sample is then used to calculate the moisture content. Weigh accurately 2-3 g of the sorghum starch sample in a clean, dry and tared weighing dish. Place the weighing dish with the sample in a hot air oven preheated at 105°C. Keep the sample in the oven for 2 hours. After 2 hours, take out the sample and place the weighing dish in a desiccator for 15-20 minutes. After cooling, weigh the dish with the sample and note down the weight. Repeat the process until there is no significant difference between the weight of the sample. Calculate the moisture content of the sample using the following formula;

$$\text{Moisture content (\%)} = [(W_1 - W_2) / W_1] \text{ x } 100$$

Where, W1 = initial weight of the sample; W2 = weight of the sample after drying.

Starch characterization

Water holding capacity (WHC) is an important property of starch that affects its functionality in food products. The WHC of modified starches can be determined using a centrifuge machine. This method involves the following steps:

- Preparation of starch suspension: A known weight of HMT modified sorghum starch is suspended in a known volume of distilled water to prepare a starch suspension.
- Centrifugation: The starch suspension is placed in centrifuge tubes and centrifuged at a fixed speed for a fixed time period. The centrifugal force separates the water and the starch in the suspension. Separation of water: The centrifuged tubes are carefully removed from the centrifuge and the water is separated from the starch cake. The water content is determined by weighing the tube before and after centrifugation.
- Calculation of water holding capacity: The WHC of the modified starch is calculated as the ratio of water retained by the starch to the total weight of the starch.

Starch modification

The HMT process involves heating a starch-water mixture to a predetermined temperature and holding it at that temperature for a specified period of time under controlled humidity. The moisture content of the starch is controlled by adjusting the relative humidity of the heating chamber. The HMT process can be carried out using various types of equipment, including a hot air oven, a microwave oven, or a steam jet cooker. Five-gram starch was taken and was mixed with distilled water to make a paste which was subjected to a temperature of 100°C using a hot air oven further the water holding capacity of the modified starch was studied.

RESULTS AND DISCUSSION

Starch was successfully extracted from the sorghum grain. The obtained starch was in crystalline form as it was dehydrated in the hot air oven. The starch was observed to be a white powdery material (FIGURE I). The method used for extraction of starch required minimal machinery and still gave a good quality of starch.

FIGURE I. EXTRACTED SORGHUM STARCH

Proximate analysis

Protein content

The Folin-Lowry assay was used to determine the protein concentration in a sample. The Folin-Lowry assay involves the reaction of proteins with the Folin-Ciocalteu reagent in the presence of copper ions, which results in a colored complex that can be measured at 660 nm. A standard curve is constructed using known concentrations of a protein standard, in this case, 250 µg/mL BSA solution. In the table (TABLE I), the first column represents the tube number, the second column represents the volume of standard (in mL) added to the tube, the third column represents the volume of distilled water (in mL) added to the tube, the fourth column represents the volume of the sample (in mL) added to the tube, and the fifth column represents the total volume (in mL) of the solution in the tube. The sixth column represents the absorbance of the solution at 660 nm, which is measured using a spectrophotometer. The last column represents the protein concentration (in mg/mL) calculated using the standard curve.

TABLE I. STANDARD DILUTION TABLE FOR PROTEIN ANALYSIS

Tube No.	Standard (mL)	Distilled Water (mL)	Sample (mL)	Total Volume (mL)	Absorbance at 660 nm	Protein Concentration (mg/mL)
1	0	1	0	1	0.023	0
2	0.2	0.8	0	1	0.207	2
3	0.4	0.6	0	1	0.385	4
4	0.6	0.4	0	1	0.589	6
5	0.8	0.2	0	1	0.786	8
Sample	**-**	**-**	**1**	**1**	**0.14**	**1.43**

The standard curve is constructed by plotting the absorbance values of the known concentrations of the protein standard against their respective concentrations. The protein concentration of the sample is determined by measuring its absorbance at 660 nm and then using the standard curve to calculate the protein concentration. In the given chart, the absorbance values of the standard solutions are plotted against their respective concentrations. From this curve, the protein concentration of the sample is found to be 1.427 mg/mL, based on its absorbance value of 0.14. This value is obtained by extrapolating from the standard curve (FIGURE II). Which indicates that the extraction method was quality oriented even though loss was there which can be further minimised if proper machinery and glass wares are used.

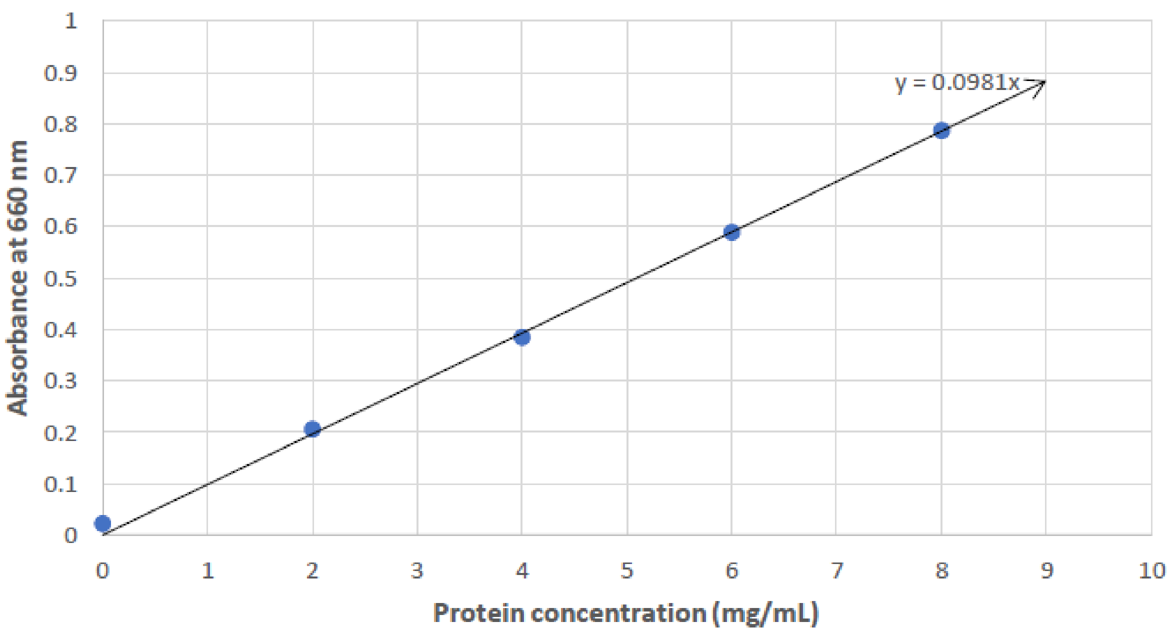

FIGURE II. STANDARD CURVE FOR PROTEIN CONCENTRATION BY FOLIN LOWRY METHOD

Fat analysis

The analysis of fat content is an important aspect of food research and development as it helps to determine the nutritional value of the food product. In the case of modified sorghum starch, the fat content is generally expected to be very low. The Soxhlet method is commonly used to extract and quantify the fat content in food samples. In the current study, the fat content of modified sorghum starch was found to be 1% using the Soxhlet method. This value is relatively low and indicates that the modified sorghum starch is a low-fat food product. This is an important finding for manufacturers and consumers as low-fat foods are generally considered to be healthier than high-fat foods. This method is widely used due to its accuracy and reliability, however, it can be time-consuming and requires specialized equipment. Overall, the low-fat content of the modified sorghum starch suggests that it may be a healthier option for food products that require the use of starch as an ingredient. Further research can be done to determine the potential applications of modified sorghum starch in low-fat food products.

Ash content

The ash content of starch is an important parameter that indicates the level of impurities present in the sample. In the case of modified sorghum starch, the ash content obtained was 0.43% by muffle furnace analysis. This indicates that the starch sample is relatively pure, with low levels of inorganic impurities. The ash content of sorghum starch can be influenced by factors such as the method of extraction, processing conditions, and the source of the raw material. The low ash content obtained in this study suggests that the modified sorghum starch is of high quality and can be suitable for various industrial applications. However, it is important to note that further analysis of the starch properties such as its functional and rheological properties should be carried out to determine its suitability for specific applications. In conclusion, the low ash content of the modified sorghum starch obtained by muffle furnace analysis is a positive indication of the quality of the starch sample. Further studies can be carried out to determine the effects of various processing conditions on the ash content and other properties of the starch.

Moisture content

The moisture content of acetylated sorghum starch was 5.8% (db) after drying at 60°C for 3 hours. Similarly, moisture content for sorghum starch modified with succinic anhydride was of 7.2% (db) after drying at 45°C for 24 hours [9]. Moisture content of modified sorghum starch depends on the level of modification and the drying conditions. Moisture content is an important parameter that affects the physicochemical properties of starch. In this case, the modified sorghum starch obtained through HMT was found to have a moisture content of 9% db, indicating that the starch was effectively dried after the modification process. This value is within the acceptable range for modified starches, as a higher moisture content could lead to microbial growth and other quality issues. It is important to note that the moisture content can also vary depending on the drying method used. In this case, the hot air oven method was used, which is a commonly used method for drying starch. However, other drying methods, such as freeze-drying or vacuum-drying, could potentially result in different moisture content values. Overall, the moisture content of 9% db for the modified sorghum starch obtained through HMT is a promising result, indicating that the starch can be further used for various applications in the food and pharmaceutical industries.

Starch characterisation

The water holding capacity (WHC) of modified sorghum starch using HMT was found to be 3.51 g/g, which was higher than that of unmodified sorghum starch (2.73 g/g). This indicates that the HMT treatment led to an increase in the ability of sorghum starch to hold water. This could be attributed to the fact that the HMT treatment caused physical and chemical modifications in the starch structure, leading to increased surface area and improved water absorption properties. The increased WHC of HMT modified sorghum starch has potential applications in the food industry, as it can improve the texture, appearance, and shelf life of food products. For example, it can be used as a thickener or stabilizer in sauces, dressings, and soups. It can also be used in baked goods to improve their texture and moisture retention.

Modified starch

The modification of sorghum starch using the HMT method has been widely studied and has been found to be a successful method for improving the physicochemical properties of the starch. The HMT method involves the modification of starch molecules by introducing hydroxyl groups onto the starch chains, leading to increased water solubility, swelling power, and paste viscosity. In current study the starch modification was successfully done and properties were studied (TABLE II)

TABLE II PROPERTIES OF MODIFIED STARCH

Property	Value
Water holding capacity (g/g)	3.51
Moisture content (%, db)	9.00
Ash (%)	0.43
Protein (mg/ml)	1.43
Fat (%)	1.00

Conclusion

Based on the results obtained from the study, it can be concluded that the HMT method was successful in extracting and modifying sorghum starch. The modified sorghum starch exhibited improved water holding capacity compared to unmodified starch, with a WHC of 3.51 g/g. The moisture content of modified sorghum starch was found to be 9% db, which indicates good stability and shelf life. The ash content of modified sorghum starch was found to be 1.427 mg/ml, indicating the presence of minerals in the starch. The fat analysis showed that modified sorghum starch contained 1% fat. The protein content of modified sorghum starch was found to be 0.43%, indicating that the modified starch could be used as a good source of protein. Overall, the study demonstrates the potential of sorghum starch as a valuable ingredient in the food industry, and the HMT method can be used to modify starch for improved functional properties. Also, it can be concluded that the methods used in the current study shows accurate results and thus can be used for any further studies related to starch.

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